1 **Cyanobacterial chemical production**

2

3 Anna E. Case and Shota Atsumi

4

5 Department of Chemistry, University of California, Davis, CA 95616, USA

6 Correspondence should be addressed to Shota Atsumi, satsumi@ucdavis.edu, Tel:

7 +1-530-752-6595, Fax: +1-530-752-8995

8

9 **Abstract**

10 The increase in global temperatures caused by rising CO₂ levels necessitates the
11 development of alternative sources of fuel and chemical. One appealing alternative
12 that has been receiving increased attention in recent years is the photosynthetic
13 conversion of atmospheric CO₂ to biofuels and chemical products using genetically
14 engineered cyanobacteria. This can help to not only provide an alternate “greener”
15 source for some of the most popular petroleum based products but it can also help
16 to reduce atmospheric CO₂. Utilizing cyanobacteria rather than plants allows for
17 reduced land requirements and reduces competition with food crops. This review
18 discusses advancements in the field since 2012 with a particular emphasis on
19 production of hydrocarbons.

20
21 **Keywords:**

22 Cyanobacteria, Photosynthesis, Metabolic Engineering, Synthetic Biology
23

1. Introduction

Many products used in daily life, from gasoline for cars to nylon and plastics, are derived from fossil fuels. In order to meet current energy and climate change challenges we must develop alternatives to fossil fuels as sources of energy and chemical production. When searching for an alternative to fossil fuels there are several important factors to consider: the efficiency of chemical production, cost, environmental impacts, and resources required (Keasling, 2012; Nielsen et al., 2014).

One promising avenue currently being explored is microbial chemical production. A great deal of work has been done to establish biofuel production in well known fermentative organisms such as yeast and *Escherichia coli* (Gronenberg et al., 2013; Jensen and Keasling, 2014; Rabinovitch-Deere et al., 2013). While this method of chemical production is a significant improvement over traditional fossil fuels, production in these host strains requires the addition of a carbon source, often sugar. This carbon source comes from plants, and therefore takes land and resources away from food crops, driving up the price of production (Tirado et al., 2010). Waste from plant crops in the form of lignocellulosics has been explored as a carbon source in these systems (Tilman et al., 2009). While advantageous in eliminating the food versus fuel land use dilemma, it is often difficult to break down these waste products into a usable form. Enzymes or high temperature and pressure conditions are often required, which adds to the cost of production (Sanderson, 2011).

Photosynthetic microorganisms offer an appealing alternative to traditional production hosts (Berla et al., 2013; Nozzi et al., 2013). With the use of a photosynthetic host it is possible to eliminate the need for added carbon and instead fix atmospheric CO₂ (Desai and Atsumi, 2013; Gronenberg et al., 2013). This also provides the added benefit of combating some of the causes of global climate change by the reduction of greenhouse gases. Without the need for added carbon, photosynthetic hosts such as algae and cyanobacteria do not require arable lands and could be grown in desert areas where they would not compete for land with food crops.

Several different cyanobacterial host species have been investigated for use in chemical production (Ducat et al., 2011; Oliver and Atsumi, 2014). A number of cyanobacterial strains have been shown to be amenable to genetic manipulation and the metabolic engineering tools available for such strains are increasing as interest in engineering cyanobacteria grows. Chemical production has already been demonstrated in cyanobacteria. In our previous review (Machado and Atsumi, 2012), we discussed some examples for cyanobacterial chemical production including isobutyraldehyde (Atsumi et al., 2009), isobutanol (Atsumi et al., 2009), 1-butanol (Lan and Liao, 2011), isoprene (Lindberg et al., 2010) and fatty acids (Liu et al., 2011). This shows the promise of cyanobacterial chemical production for wide scale use. However, there are several challenges to overcome before the goal of large-scale chemical production from cyanobacteria can be realized (Nozzi et al., 2013). These challenges include establishing tight control of gene expression, improving titers and removing bottlenecks, and expanding beyond established

pathways (Boyle and Silver, 2012; Camsund and Lindblad, 2014; Marcheschi et al., 2013). This review discusses the progress made since 2012 (Machado and Atsumi, 2012) towards overcoming these obstacles with a focus on hydrocarbon production (Table 1).

2. New Production Pathways in Cyanobacteria

One bottleneck in the broad use of cyanobacterial chemical production is the limited number of chemicals that can be produced. In recent years a great deal of work has been done to broaden the diversity of these chemicals and expand on the utility of cyanobacteria (Table 1).

2.1 Alcohols and diols

A wide variety of pathways for the production of industrially important chemicals have been developed in recent years. A prominent area of chemical production in cyanobacteria is the production of alcohols and diols. Alcohols and diols are both popular for use as biofuel candidates. Alcohols that have already been produced in engineered cyanobacteria include 1-butanol and isobutanol (Atsumi et al., 2009; Lan and Liao, 2011).

As mentioned in our previous review (Machado and Atsumi, 2012), production of isobutanol is reliant on the valine biosynthesis pathway (Atsumi et al., 2009). The intermediate 2-ketoisovalerate is funneled away from valine production and redirected to isobutanol through the activities of the heterologous enzymes 2-ketoacid decarboxylase and alcohol dehydrogenase (Figure 1). Similar approaches

have been applied to produce 2-methyl-1-butanol in cyanobacteria (Shen and Liao, 2012). 2-Methyl-1-butanol is derived from isoleucine biosynthesis and instead utilizes the intermediate 2-keto-3-methylvalerate (Figure 1). A 2-ketoacid decarboxylase and alcohol dehydrogenase along with the citramalate pathway were utilized to produce 2-methyl-1-butanol in *Synechococcus elongatus* PCC7942 (7942) with a final titer of 200 mg/L (Shen and Liao, 2012).

Isopropanol is of great interest because it can be readily converted into propylene and then to polypropylene, an important industrial material (Kusakabe et al., 2013). The addition of the isopropanol pathway (Figure 2) from *Clostridium* (an acetyl transferase, acetyl-CoA acetyl transferase, acetoacetate decarboxylase, and a secondary alcohol dehydrogenase) in 7942 produced 27 mg/L after 9 days (Kusakabe et al., 2013). Building on this work by optimization of light conditions and buffering of the growth media helped to achieve an increase in production from 27 mg/L to 146 mg/L (Hirokawa et al., 2015).

Our lab has demonstrated that the production of diols is also possible in cyanobacteria, such as 2,3-butanediol (23BDO) (Oliver et al., 2013). Starting from pyruvate, 23BDO production can be achieved through the addition of three enzymes, acetolactate synthase, acetolactate decarboxylase, and an alcohol dehydrogenase (Figure 1). Acetolactate synthase catalyzes the condensation of two pyruvate molecules into one acetolactate, which is then decarboxylated to acetoin by acetolactate decarboxylase. The final step is the reduction of acetoin to 23BDO by a secondary alcohol dehydrogenase. We demonstrated production in 7942 and final titers reached 2.4 g/L after 20 days (Oliver et al., 2013). Similarly,

Synechocystis sp. PCC 6803 (6803) was engineered to produce 23BDO by another group, with a final titer of 430 mg/L (Savakis et al., 2013).

Cyanobacteria's natural propensity to produce fatty alcohols (Figure 2) has been improved by expression of a non-native fatty-acyl coA reductase gene and the deactivation of the native acyl-ACP reductase and aldehyde deformylating oxygenase (Yao et al., 2014). The modifications improved fatty alcohol production to 2.9 mg/g dry cell weight (DCW), up from the previously reported 0.8 mg/g DCW (Yao et al., 2014).

2.2 Hydroxy acids

The global market for plastics is expected to exceed 654 billion dollars by the year 2020 (James, 2014), and plastic are currently produced primarily from petroleum sources. Therefore, there is significant incentive to move away from petroleum sources for the production of plastics and polymers. Toward that end the production of 3 different plastic precursor molecules in cyanobacteria have been established (Figure 2) (Lan et al., 2015; Wang et al., 2013a; Zhang et al., 2015). First, 3-hydroxybutyrate (3HB) was produced in 6803. 3HB production had been previously demonstrated in *E. coli* by the catalysis of two enzymatic steps (Gao et al., 2002). The first step requires the condensation of two acetyl-CoA molecules by a thiolase to make acetoacetyl-CoA. This is followed by the reduction of acetoacetyl-CoA by an acetoacetyl-CoA reductase leading to formation of 3HB. Titrers reached 533 mg/L after 21 days (Wang et al., 2013b). Second, two different routes to produce 3-hydroxypropanoic acid (3HP) in 7942 have been explored (Lan et al.,

2015). One pathway is malonyl-CoA dependent and produced 3HP at a maximal titer of 665 mg/L. The second pathway is β -alanine dependent and achieved 186 mg/L 3HP (Lan et al., 2015). Last, poly-3-hydroxybutyrate (P3HB) and poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P3H4HB) were produced in *Synechococcus* sp. PCC7002 (7002) through the introduction of a three or six-step pathway, respectively, from *Chlorogloeopsis fritschii* PCC 9212 (Zhang and Bryant, 2015). The gene cluster *phaABEC*, which encodes for an acetyl-CoA transferase, an acetyl-CoA reductase and a poly(R)-hydroxy alkanoic acid synthase, was introduced on a high copy plasmid (pAQ1) to convert acetyl-CoA to P3HB. Three additional enzymes, 4-hydroxybutyryl-CoA transferase, 4-hydroxybutyrate dehydrogenase, and 2-oxoglutarate decarboxylase imparted production of the co-polymer P3H4HB. The precursor to the co-polymer, 4-hydroxybutyrate (P4HB), was produced from succinic semialdehyde via the citric acid cycle. Yields achieved were 5% total cell dry weight P3HB and 4.5% of the copolymer P3H4HB (Zhang et al., 2015).

2.3 Alkanes

Production of a short chain alkene, ethylene, is also crucial to reduce dependency on petrochemical feedstocks. Ethylene is the most widely produced petrochemical feedstock on earth, and its production is the largest CO₂ emitting process in the chemical industry (McCoy et al., 2006). An engineered 6803 has been shown to convert CO₂ to ethylene (Figure 2) (Ungerer et al., 2012). This was accomplished by first installing an ethylene forming enzyme (EFE) from *Pseudomonas syringae* pv. phaseolicola (Ungerer et al., 2012). The stability of the

enzyme was improved through site directed mutagenesis to achieve a final production titer of 5.7 mL/L culture/h (Ungerer et al., 2012). Ethylene production in 6803 was also achieved through the installation of a His-tagged EFE to a rate of 0.2 mL/L culture/h. (Guerrero et al., 2012).

Longer chain alkanes are promising biofuel candidates (Lennen and Pfleger, 2013). Production of the long chain alkane heptadecane has been achieved in the marine cyanobacterium *Synechococcus* sp. NKBG15041c (Figure 2) (Yoshino et al., 2015). There are two pathways commonly seen in cyanobacteria for alkane production, an acyl-acyl-carrier protein (ACP) with a reductase/aldehyde-deformylating oxygenase pathway (AAR/ADO) and an α -olefin synthase pathway (OLS). The two pathways had never been co-expressed in the same organism (Yoshino et al., 2015). The strain was shown to naturally contain the OLS pathway, resulting in the production of nonadecadiene and 1-nonadecene. The ACP, AAR/ADO pathway was added to the strain to facilitate production of heptadecane. The highest production level of heptadecane achieved was 4 μ g/g DCW, nonadecadiene and 1-nonadecene represented the majority of the alkanes produced in the strain but amounts were not quantified (Yoshino et al., 2015).

2.4 Fatty acids

Free fatty acids have possible applications for use in the production of biodiesels. Previously, production of fatty acids was established in *E. coli* and has also been demonstrated in two cyanobacterial species (7942 and 6803) (Figure 2), though some negative physiological effects including reduced viability, altered cell

membrane composition, increased reactive oxygen species and reduced photosynthetic yields had been reported (Lennen et al., 2011; Liu et al., 2011; Ruffing, 2013). Moving production into 7002 showed similar titers to that in 7942 and 6803 (>130mg/L) but with reduced physiological effects as demonstrated by an increased growth rate and photosynthetic yield (Ruffing, 2014).

2.5 Sucrose

As an alternative approach to producing biofuels in cyanobacteria, another strategy that has been explored is the use of cyanobacteria as a sugar feedstock for biofuels production in other fermentative organisms (Du et al., 2013; Ducat et al., 2012). Cyanobacteria naturally accumulate sucrose under salt-stress conditions (500 mM NaCl) with the addition of CscB, a sucrose exporter from *E. coli*, and the deletion of ADP-glucose pyrophosphorylase (*glgC*) and invertase (*invA*) genes to redirect carbon flux away from glycogen production (Figure 3). The combined modifications resulted in a maximum sucrose productivity of 36 mg/L/h (Ducat et al., 2012). To expand sucrose production, three different cyanobacterial species (7942, 6803, and *Anabaena* sp. PCC 7120 (7120)) were also investigated for their ability to produce sucrose (Du et al., 2013). Further genetic modifications were made to these strains to increase sucrose production, including the overexpression of the sucrose phosphate synthase, sucrose phosphate phosphatase, and UDP-glucose pyrophosphorylase genes, and the knockdown of the glucosylglycerol phosphate synthase gene. Overall, a four-fold increase in sucrose production was

observed over wild type strains, with final titers of 140 mg/L, and rates of 5-6 mg/L/h (Du et al., 2013).

2.6 Glucosylglycerol

Glucosylglycerol (GG) has applications in pharmaceuticals and cosmetics because of its protein stabilizing and antioxidant properties (Sawangwan et al., 2010). 6803 naturally produce GG in response to osmotic stress (Figure 3) (Klahn et al., 2010). This natural production was enhanced by disrupting two GG uptake genes and a GG repressor gene (Tan et al., 2015). GG was produced to a titer of 250 mg/L after 8 days (Tan et al., 2015).

2.7 Isoprenoid

Squalene is a 30-carbon isoprenoid commonly used in cosmetics and vaccines (Figure 4) (Englund et al., 2014). The removal of a squalene hypocyclase in 6803 resulted in the accumulation of squalene up to 0.7 mg/OD₇₅₀/L (Englund et al., 2014). Additionally, no negative growth effects were observed. Another compound with pharmaceutical applications, forskolin, is a diterpenoid (Englund et al., 2015). Current production of forskolin requires extraction of the chemical from plants because chemical synthesis is too complex to be economically viable (Pateraki et al., 2014). To achieve forskolin production in cyanobacteria, two diterpene synthases, CfTPS2 and CfTPS3, from *Chlorogloeopsis forskohlii* were introduced into 6803. Together these two enzymes catalyze the conversion of geranylgeranyl diphosphate to 13R-manoyl oxide (Figure 4), a precursor to

forskolin. The engineered strain produced 13R-manoyl oxide at 0.5 mg/g DCW (Englund et al., 2015).

2.8 Farnesene

Building block chemicals that can be readily converted into other chemicals are of great interest in biological production. Farnesene is commonly used as a building block for a large variety of different products used in everyday life (Halfmann et al., 2014). The utilization of the endogenous non-mevalonate pathway in 7120 enabled the strain to produce farnesene (Figure 4). The engineered strain produced 70 µg/L/OD/day farnesene (Halfmann et al., 2014).

3. Improving production in previously established pathways

In addition to finding new pathways for chemical production, it will also be necessary to increase titers and productivities of the chemicals that have already been produced. There are three main avenues that have been explored in recent years to increase target chemical production from cyanobacteria: The manipulation of carbon flux, additional carbon sources, and controlling gene expression.

3.1 Manipulating carbon flux

Manipulating carbon flux has the capacity to not only increase the production of the desired product but also reduce non-desired side products. One mechanism to increase carbon flux is to increase production of precursors, which creates a “pull” toward the desired product, and can help increase the carbon flow through

the pathway. For example, isoprene production in cyanobacteria had been previously demonstrated with limited productivities (Lindberg et al., 2010) so attempts were made to improve isoprene production by increasing carbon partitioning toward isoprene (Bentley et al., 2014). This was achieved by overexpression of the mevalonic acid pathway enzymes to boost production of the isoprene precursors isopentenyl-diphosphate and dimethylallyl-diphosphate. This altered carbon partitioning resulted in a titer of 250 μ g isoprene/g DCW, which represented a 2.5 fold increase (Bentley et al., 2014). Additionally, our lab has been able to demonstrate increased 23BDO production through increased carbon flux to 23BDO (Oliver and Atsumi, 2015). The overexpression of the native genes responsible for pyruvate production from the Calvin cycle resulted in increased carbon flux to the desired product. Overall a 1.8-fold increase in carbon fixation rate was observed, 4.3 mM/gDCW/h (Oliver and Atsumi, 2015).

Another approach to increasing carbon flux to a desired product is through the removal of alternate pathways. Glycogen represents a significant carbon storage mechanism in cyanobacteria that naturally redirects carbon flow away from desired products (Carrieri et al., 2010; Carrieri et al., 2012; Grundel et al., 2012). This concept of rerouting carbon from glycogen storage has been applied to lactate production in 6803 (Figure 1) (van der Woude et al., 2014). The pathway for glycogen production was deleted from the strain, and a two-fold increase in lactate production was observed (van der Woude et al., 2014). However, deletion of the glycogen pathway also yields observable growth deficiencies (Jacobsen et al., 2011) thought to be a result of imbalanced carbon and/or redox metabolism (Li et al.,

2014). To avoid this issue, the use of isobutanol production as a metabolic sink was investigated (Li et al., 2014). Partial growth restoration was observed with expression of the isobutanol pathway in 7942, however, that restoration was thought to be limited by isobutanol production (Li et al., 2014). Carbon analysis revealed that 52% of total fixed carbon was directed toward isobutanol production, a 2.5 fold increase over previous results lacking the glycogen deletion (Li et al., 2014).

Increased carbon flux can also be achieved through increased efficiency of the enzymes in the target chemical production pathway. Increasing the turnover rate of one or more key enzymes in the pathway can cause an overall increase in carbon flow through the pathway. In one such case, lactic acid production in 6803 (Figure 1) was improved by optimizing gene expression, increasing carbon flux and eliminating competing pathways (Angermayr et al., 2014). The production was further increased by optimizing lactate dehydrogenase, a key enzyme in the pathway, through site directed mutagenesis. Eventually, carbon partitioning to lactic acid was increased from 5% to over 50% (Angermayr et al., 2014).

One of the most well know biofuels is ethanol, but current production routes are heavily reliant on corn and other food crops to supply the sugar precursors (Tilman et al., 2009). As an alternative to funneling food crops toward biofuel production, significant work has been done to improve the production levels of a previously established pathway for the production of ethanol in cyanobacteria (Figure 1) (Deng and Coleman, 1999). By balancing cofactors and enzyme activities

to achieve the optimal conditions for ethanol production in 6803; the final titer achieved was 2.3 g/L (Luan et al., 2015).

It is also possible to alter carbon flow by altering culture conditions. In one such instance, salt stress (1.24M NaCl) increased hydrogen and ethanol production in 7942 and 7002, respectively (Carrieri et al., 2010).

3. 2 Additional carbon sources

One of the primary advantages of using cyanobacteria for biofuel production is their ability to convert atmospheric CO₂ into valuable products. However, manipulating cyanobacteria to allow them to utilize additional carbon sources can help to boost overall production levels and increase the utility of this system.

We have successfully demonstrated the ability of an obligate photoautotrophic cyanobacterium (7942) to utilize sugar by the addition of a sugar transporter (McEwen et al., 2013). This allowed for continuous growth under diurnal light conditions. The strain was able to utilize glucose, sucrose, and xylose under dark conditions while still maintaining its ability to fix CO₂ under light conditions. A different transporter was required for utilization of each sugar. For glucose, a galactose:H⁺ symporter, encoded by *galP* from *E. coli*, showed the greatest and most consistent growth improvement. The addition of two enzymes, a sucrose transporter and a fructokinase (*cscB* and *cscK*) allowed the strain to efficiently utilize sucrose for improved growth under both light and dark conditions. The gene cassette *xylEAB* from *E. coli* imparted the ability to successfully utilize xylose for growth. *xylE* encodes a xylose transporter from *E. coli*, but the expression of *xylE*

alone was toxic to cells, with a significantly reduced growth rate compared to wild type, so two additional genes for a xylose isomerase (*xylA*) and xylulokinase (*xylB*) were required. 7942 lacks these two enzymes responsible for initiating the breakdown of xylose so all three genes were required for successful utilization of xylose (McEwen et al., 2013). This work was further expanded upon by demonstrating 23BDO production from high density engineered 7942 strains incubated under continuous light conditions with either glucose or xylose (McEwen et al., 2016). From both the glucose (*galP*) and xylose (*xylEAB*) strains, a significant increase in both biomass accumulation (93% and 179% respectively), and 23BDO production (202% and 453% respectively) was observed. A maximum 23BDO titer of 3 g/L after 10 days was achieved from the glucose strain (McEwen et al., 2016). Interestingly, under diurnal light conditions (12h light/ 12h dark) growth was slower during the dark phase but production remained constant. Under constant dark conditions, sugar was required for both growth and 23BDO production. In the presence of glucose, 0.16 g/L 23BDO was produced, and in the presence of xylose 0.18 g/L 23BDO was produced in 72 h (McEwen et al., 2016). This work expanded the production potential of cyanobacteria by counteracting the issue of light availability during industrial scale production.

6803 can naturally consume glucose. Isobutanol production in 6803 has been demonstrated under mixotrophic conditions (Varman et al., 2013a). The addition of glucose increased the isobutanol titer from 90 mg/L to 114 mg/L. Further improvement came from the use of oleyl alcohol as a solvent trap to relieve toxicity and increased the isobutanol titer to 298 mg/L (Varman et al., 2013a).

This approach of improving sugar consumption in cyanobacteria has also been investigated for ethylene production in 6803 (Lee et al., 2015). Two genes for xylose utilization from *E. coli* (*xylAB*) were installed into the ethylene producing 6803 strain, and led to an overall boost in production over the non-xylose consuming strain. Final ethylene titers from high density cultures ($OD_{730} \sim 5.3$), where light is limiting, reached 11.3 mL/L culture, and a rate of 0.9 mL/L culture/h.

While the use of sugar as an additional carbon source is very appealing, another alternate that is arguably even more desirable are carbon sources found in waste water such as acetate. Imparting the ability to utilize acetate would not only bolster target chemical production but could also help remove contaminants from waste streams. A strain of 6803 has been developed to produce D-lactic acid by simultaneous use of acetate and CO₂ (Varman et al., 2013c). Moderate improvements to production were achieved through codon optimization of the *ldh* gene (from *Bacillus subtilis*) and installation of a soluble transhydrogenase gene *sth* (from *Pseudomonas aeruginosa*). The *sth* gene was installed to increase the NADH pool and relieve it as a bottleneck. D-lactic acid production was further enhanced by the addition of acetate to the cultures, though C¹³ analysis revealed that acetate primarily contributed to biomass and not lactic acid production. The final D-lactic acid titer achieved was 1.1 g/L without the addition of acetate and 2.2 g/L with the addition of acetate (Varman et al., 2013c).

3.3 Controlling gene expression

A key factor in achieving stable gene expression in cyanobacteria is the identification and development of sites in the genome for integration of exogenous genes via homologous recombination into either the chromosome or endogenous plasmids. A platform for high-level gene expression in 7002 has been established (Xu et al., 2011). Six endogenous plasmids were adapted for the expression of non-native genes. Non-native genes were successfully introduced and expressed without disturbing essential genes (integration points called “neutral sites” (Xu et al., 2011)). These plasmids serve as an expression platform for further exploration of chemical synthesis in 7002, and add to the metabolic engineering tools available (Xu et al., 2011).

In another study, new neutral sites were identified that increase the sites for transformation of 6803 (Ng et al., 2015). From transcriptome sequencing, two new sites that do not express transcripts and thus would not disturb natural cell functions were identified. One site was on the chromosome and the other on an endogenous plasmid. The *yfp* gene encoding a yellow fluorescence protein was incorporated in both sites, and the site on the plasmid showed 14-fold higher expression than the site on the chromosome (Ng et al., 2015).

It is essential to be able to control gene expression in an inducible manner. Achieving high levels of production often relies upon being able to grow cells to an acceptable density prior to induction, limiting growth deficiencies caused by chemical production. Several different approaches for gene expression control have been investigated at both the transcriptional and translational level. A theophylline dependent riboswitch (Topp et al., 2010), which constitutes translational control of

gene expression, was utilized to control gene expression in 7942 (Nakahira et al., 2013). The best riboswitch construct demonstrated 190-fold induction with a theophylline range from 0 to 2 mM. Riboswitches can offer an additional layer of gene control for production constructs (Nakahira et al., 2013) or can stand on their own to control gene expression. A series of riboregulators, a form of post-transcriptional gene control composed of two parts, a cis-prepressed mRNA (crRNA) and a trans-activating RNA (taRNA), (Isaacs et al., 2004) were constructed in 6803 (Abe et al., 2014). The crRNA contains the ribosomal binding site and forms a stem-loop structure, preventing translational initiation, while the taRNA is able to bind to the internal stem-loop sequence and promote gene expression. This system was tested in 6803 using green fluorescent protein as a reporter, and proved effective at controlling gene expression with a 13-fold increase in *gfp* expression upon induction (Abe et al., 2014). No growth detriment was observed. A riboregulator was also adapted for use in 7002 (Zess et al., 2016). When used together a 59% attenuation of target genes was observed (Zess et al., 2016).

At the transcriptional level, promoter systems have been adapted from other host organisms and optimized for use in cyanobacteria (Markley et al., 2015). For instance, *E. coli* IPTG inducible promoters such as pLacO1 (Lutz and Bujard, 1997) and pTrc (Amann et al., 1988) have been optimized for controlling gene expression in 7002 (Markley et al., 2015). The best promoter system, termed cLac143, showed a 48-fold dynamic range (Markley et al., 2015).

A TetR-regulated (anhydrotetracycline [aTc]-dependent) promoter has been developed in 6803 (Huang and Lindblad, 2013). The promoter provided a wide

411 range of transcriptional regulation with 239-fold induction under dark conditions
412 and 290-fold induction under light conditions with 10 $\mu\text{g/mL}$ aTc. Altering a few
413 base pairs between the -10 box and the transcriptional start site resulted a wide
414 range of induction (Huang and Lindblad, 2013). This system was later adapted to
415 create an aTc inducible promoter in 7002. This promoter showed a 32-fold
416 induction (Markley et al., 2015).

417 The ribosomal binding site can also play a large role in the level of gene
418 expression in an inducible system. We have attempted to improve production of
419 23BDO in 7942 by optimizing the ribosomal binding site of each of the three
420 transcripts in the 23BDO pathway (Oliver et al., 2014). There was not enough
421 information available to take a rational design approach so we utilized a
422 combinatorial approach with 4 different RBS sequences. We observed a broad
423 range in 23BDO production, from 207 mg/L in the weakest construct to 496 mg/L in
424 the strongest construct. This demonstrated the importance of regulating and
425 optimizing gene expression for achieving high production (Oliver et al., 2014).

426 To achieve dynamic expression range in 7002, an RBS library has been
427 designed and screened (Markley et al., 2015). The RBS sequence was modified
428 using an RBS library calculator (Salis et al., 2009) to create a library of 8 possible
429 RBS sequences with a predicted expression range of 213-fold. In practice, a dynamic
430 range of 30-fold was observed for the library (Markley et al., 2015). The RBS library
431 successfully altered transcript level and increased the dynamic range of the system
432 but prediction of expression levels is still difficult.

We have also explored other routes to improve gene expression and regulation in cyanobacteria (Nozzi and Atsumi, 2015). Previous production of 23BDO in cyanobacteria had been established with poor control of production (Oliver et al., 2013). The efficacy of the promoter pLacO1, which was used for expression of the 23BDO pathway, was investigated using a green fluorescent protein as a reporter. Constructs lacking pLacO1 revealed native constitutive promoters in two of the genes in the 23BDO pathway (*alsS* and *adh*), and gene order was found to be critical for controlling gene expression. By optimizing gene order we were able to show improvement in production and reduced leakiness. This work added to the metabolic engineering “toolbox” for this strain by demonstrating that pLacO1 does control gene expression well and that gene order is critical in achieving gene repression (Nozzi and Atsumi, 2015).

4. Conclusion

In the last few years a number of significant advancements have been made toward engineering cyanobacteria for the production of valuable chemicals (Table 1). New pathways have been developed for the production of alcohols and diols, alkanes, polymers, and even products with pharmaceutical applications such as GG or squalene. In addition to a drastic broadening of target chemicals possible in these host organisms, the field has also seen advancements in chemical production levels driven by the manipulation of carbon flux, use of additional carbon sources, and improved control of gene expression. Taken together the works described herein represent a demonstration of the potential of cyanobacteria as a host organism.

However, in order for cyanobacteria to become industrially relevant, further work needs to be done to increase yields and productivities. Another challenge that will need to be addressed is the gap between lab scale and industrial scale production. At an industrial scale new factors come into play such as light availability. Utilization of sugar and acetate can help bridge this gap but further work will be required for successful scale up. Another limiting factor is the availability of freshwater. Moving toward use of cyanobacterial strains with a higher salt tolerance, or developing systems with lower water requirements such as biofilms can aide in overcoming this challenge. The work done in recent years has served to drastically improve the feasibility of using cyanobacteria for broad scale chemical production but more work will still need to be done.

Acknowledgments

We would like to thank Christine A. Rabinovitch-Deere and Nicole E. Nozzi for critical reading of the manuscript. This work was supported by the National Science Foundation CBET-1349663.

References

- Abe, K., Sakai, Y., Nakashima, S., Araki, M., Yoshida, W., Sode, K., Ikebukuro, K., (2014) Design of riboregulators for control of cyanobacterial (*Synechocystis*) protein expression. *Biotechnol Lett* 36, 287-294.
- Amann, E., Ochs, B., Abel, K.-J., (1988) Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. *Gene* 69, 301-315.
- Angermayr, S.A., van der Woude, A.D., Correddu, D., Vreugdenhil, A., Verrone, V., Hellingwerf, K.J., (2014) Exploring metabolic engineering design principles for the photosynthetic production of lactic acid by *Synechocystis* sp. PCC6803. *Biotechnol Biofuels* 7, 1-15.
- Atsumi, S., Higashide, W., Liao, J.C., (2009) Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. *Nat Biotechnol* 27, 1177-1180.
- Bentley, F.K., Zurbriggen, A., Melis, A., (2014) Heterologous expression of the mevalonic acid pathway in cyanobacteria enhances endogenous carbon partitioning to isoprene. *Mol Plant* 7, 71-86.
- Berla, B.M., Saha, R., Immethun, C.M., Maranas, C.D., Moon, T.S., Pakrasi, H.B., (2013) Synthetic biology of cyanobacteria: unique challenges and opportunities. *Front Microbiol* 4, 246.
- Boyle, P.M., Silver, P.A., (2012) Parts plus pipes: synthetic biology approaches to metabolic engineering. *Metab Eng* 14, 223-232.
- Camsund, D., Lindblad, P., (2014) Engineered transcriptional systems for cyanobacterial biotechnology. *Front Bioeng Biotechnol* 2, 40.

495 Carrieri, D., Momot, D., Brasg, I.A., Ananyev, G., Lenz, O., Bryant, D.A., Dismukes, G.C.,
 496 (2010) Boosting autofermentation rates and product yields with sodium stress
 497 cycling: application to production of renewable fuels by cyanobacteria. *Appl Environ*
 498 *Microbiol* 76, 6455-6462.

499 Carrieri, D., Paddock, T., Maness, P.C., Seibert, M., Yu, J.P., (2012) Photo-catalytic
 500 conversion of carbon dioxide to organic acids by a recombinant cyanobacterium
 501 incapable of glycogen storage. *Energy Environ Sci* 5, 9457-9461.

502 Deng, M.D., Coleman, J.R., (1999) Ethanol synthesis by genetic engineering in
 503 cyanobacteria. *Appl Environ Microbiol* 65, 523-528.

504 Desai, S.H., Atsumi, S., (2013) Photosynthetic approaches to chemical biotechnology.
 505 *Curr Opin Biotechnol* 24, 1031-1036.

506 Du, W., Liang, F., Duan, Y., Tan, X., Lu, X., (2013) Exploring the photosynthetic
 507 production capacity of sucrose by cyanobacteria. *Metab Eng* 19, 17-25.

508 Ducat, D.C., Avelar-Rivas, J.A., Way, J.C., Silver, P.A., (2012) Rerouting carbon flux to
 509 enhance photosynthetic productivity. *Appl Environ Microbiol* 78, 2660-2668.

510 Ducat, D.C., Way, J.C., Silver, P.A., (2011) Engineering cyanobacteria to generate
 511 high-value products. *Trends Biotechnol* 29, 95-103.

512 Englund, E., Andersen-Ranberg, J., Miao, R., Hamberger, B., Lindberg, P., (2015)
 513 Metabolic engineering of *Synechocystis* sp. PCC 6803 for production of the plant
 514 diterpenoid manoyl oxide. *ACS Synth Biol* 4, 1270-1278.

515 Englund, E., Pattanaik, B., Ubhayasekera, S.J., Stensjo, K., Bergquist, J., Lindberg, P.,
 516 (2014) Production of squalene in *Synechocystis* sp. PCC 6803. *PLoS One* 9, e90270.

517 Gao, H.J., Wu, Q., Chen, G.Q., (2002) Enhanced production of D-(-)-3-hydroxybutyric
 518 acid by recombinant *Escherichia coli*. FEMS Microbiol Lett 213, 59-65.
 519 Gronenberg, L.S., Marcheschi, R.J., Liao, J.C., (2013) Next generation biofuel
 520 engineering in prokaryotes. Curr Opin Chem Biol 17, 462-471.
 521 Grundel, M., Scheunemann, R., Lockau, W., Zilliges, Y., (2012) Impaired glycogen
 522 synthesis causes metabolic overflow reactions and affects stress responses in the
 523 cyanobacterium *Synechocystis* sp. PCC 6803. Microbiology 158, 3032-3043.
 524 Guerrero, F., Carbonell, V., Cossu, M., Correddu, D., Jones, P.R., (2012) Ethylene
 525 synthesis and regulated expression of recombinant protein in *Synechocystis* sp. PCC
 526 6803. PLoS One 7.
 527 Halfmann, C., Gu, L., Gibbons, W., Zhou, R., (2014) Genetically engineering
 528 cyanobacteria to convert CO₂, water, and light into the long-chain hydrocarbon
 529 farnesene. Appl Microbiol Biotechnol 98, 9869-9877.
 530 Hirokawa, Y., Suzuki, I., Hanai, T., (2015) Optimization of isopropanol production by
 531 engineered cyanobacteria with a synthetic metabolic pathway. J Biosci Bioeng 119,
 532 585-590.
 533 Huang, H.H., Lindblad, P., (2013) Wide-dynamic-range promoters engineered for
 534 cyanobacteria. J Biol Eng 7, 10.
 535 Isaacs, F.J., Dwyer, D.J., Ding, C., Pervouchine, D.D., Cantor, C.R., Collins, J.J., (2004)
 536 Engineered riboregulators enable post-transcriptional control of gene expression.
 537 Nat Biotechnol 22, 841-847.

538 Jacobsen, J.H., Rosgaard, L., Sakuragi, Y., Frigaard, N.U., (2011) One-step plasmid
 539 construction for generation of knock-out mutants in cyanobacteria: studies of
 540 glycogen metabolism in *Synechococcus* sp. PCC 7002. Photosynth Res 107, 215-221.
 541 James, S., (2014) Global Plastics Market, Size & Forecast to 2020 by Grand View
 542 Research, Inc.
 543 Jensen, M.K., Keasling, J.D., (2014) Recent applications of synthetic biology tools for
 544 yeast metabolic engineering. FEMS Yeast Res 15, 1-10.
 545 Justin Ungerer, L.T., Mark Davis, Maria Ghirardi, Pin-Ching Maness and Jianping Yu,
 546 (2012) Sustained photosynthetic conversion of CO₂ to ethylene in recombinant
 547 cyanobacterium *Synechocystis* 6803. Energy Environ Sci 5, 8998–9006.
 548 Keasling, J.D., (2012) Synthetic biology and the development of tools for metabolic
 549 engineering. Metab Eng 14, 189-195.
 550 Klahn, S., Hohne, A., Simon, E., Hagemann, M., (2010) The gene *ssl3076* encodes a
 551 protein mediating the salt-induced expression of *ggpS* for the biosynthesis of the
 552 compatible solute glucosylglycerol in *Synechocystis* sp. strain PCC 6803. J Bacteriol
 553 192, 4403-4412.
 554 Kusakabe, T., Tatsuke, T., Tsuruno, K., Hirokawa, Y., Atsumi, S., Liao, J.C., Hanai, T.,
 555 (2013) Engineering a synthetic pathway in cyanobacteria for isopropanol
 556 production directly from carbon dioxide and light. Metab Eng 20, 101-108.
 557 Lan, E.I., Chuang, D.S., Shen, C.R., Lee, A.M., Ro, S.Y., Liao, J.C., (2015) Metabolic
 558 engineering of cyanobacteria for photosynthetic 3-hydroxypropionic acid
 559 production from CO₂ using *Synechococcus elongatus* PCC 7942. Metab Eng 31, 163-
 560 170.

561 Lan, E.I., Liao, J.C., (2011) Metabolic engineering of cyanobacteria for 1-butanol
 562 production from carbon dioxide. *Metab Eng* 13, 353-363.

563 Lee, T.C., Xiong, W., Paddock, T., Carrieri, D., Chang, I.F., Chiu, H.F., Ungerer, J., Juo,
 564 S.H., Maness, P.C., Yu, J., (2015) Engineered xylose utilization enhances bio-products
 565 productivity in the cyanobacterium *Synechocystis* sp. PCC 6803. *Metab Eng* 30, 179-
 566 189.

567 Lennen, R.M., Kruziki, M.A., Kumar, K., Zinkel, R.A., Burnum, K.E., Lipton, M.S.,
 568 Hoover, S.W., Ranatunga, D.R., Wittkopp, T.M., Marner, W.D., 2nd, Pfleger, B.F.,
 569 (2011) Membrane stresses induced by overproduction of free fatty acids in
 570 *Escherichia coli*. *Appl Environ Microbiol* 77, 8114-8128.

571 Lennen, R.M., Pfleger, B.F., (2013) Microbial production of fatty acid-derived fuels
 572 and chemicals. *Curr Opin Biotechnol* 24, 1044-1053.

573 Li, X., Shen, C.R., Liao, J.C., (2014) Isobutanol production as an alternative metabolic
 574 sink to rescue the growth deficiency of the glycogen mutant of *Synechococcus*
 575 *elongatus* PCC 7942. *Photosynth Res* 120, 301-310.

576 Lindberg, P., Park, S., Melis, A., (2010) Engineering a platform for photosynthetic
 577 isoprene production in cyanobacteria, using *Synechocystis* as the model organism.
 578 *Metab Eng* 12, 70-79.

579 Liu, X., Sheng, J., Curtiss, R., 3rd, (2011) Fatty acid production in genetically modified
 580 cyanobacteria. *Proc Natl Acad Sci U S A* 108, 6899-6904.

581 Luan, G., Qi, Y., Wang, M., Li, Z., Duan, Y., Tan, X., Lu, X., (2015) Combinatory strategy
 582 for characterizing and understanding the ethanol synthesis pathway in
 583 cyanobacteria cell factories. *Biotechnol Biofuels* 8, 184.

584 Lutz, R., Bujard, H., (1997) Independent and tight regulation of transcriptional units
 585 in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements.
 586 Nucleic Acids Res 25, 1203-1210.
 587 Machado, I.M., Atsumi, S., (2012) Cyanobacterial biofuel production. J Biotechnol
 588 162, 50-56.
 589 Marcheschi, R.J., Gronenberg, L.S., Liao, J.C., (2013) Protein engineering for
 590 metabolic engineering: current and next-generation tools. Biotechnol J 8, 545-555.
 591 Markley, A.L., Begemann, M.B., Clarke, R.E., Gordon, G.C., Pflieger, B.F., (2015)
 592 Synthetic biology toolbox for controlling gene expression in the cyanobacterium
 593 *Synechococcus* sp. strain PCC 7002. ACS Synth Biol 4, 595-603.
 594 McCoy, M., Reisch, M., Tullo, A., (2006) Production: Growth Is the Norm. Chemical &
 595 Engineering News 84, 59-68.
 596 McEwen, J.T., Kanno, M., Atsumi, S., (2016) 2,3 Butanediol production in an obligate
 597 photoautotrophic cyanobacterium in dark conditions via diverse sugar
 598 consumption. Metab Eng 36, 28-36.
 599 McEwen, J.T., Machado, I.M., Connor, M.R., Atsumi, S., (2013) Engineering
 600 *Synechococcus elongatus* PCC 7942 for continuous growth under diurnal conditions.
 601 Appl Environ Microbiol 79, 1668-1675.
 602 Nakahira, Y., Ogawa, A., Asano, H., Oyama, T., Tozawa, Y., (2013) Theophylline-
 603 dependent riboswitch as a novel genetic tool for strict regulation of protein
 604 expression in cyanobacterium *Synechococcus elongatus* PCC 7942. Plant Cell Physiol
 605 54, 1724-1735.

606 Ng, A.H., Berla, B.M., Pakrasi, H.B., (2015) Fine-tuning of photoautotrophic protein
 607 production by combining promoters and neutral sites in the cyanobacterium
 608 *Synechocystis* sp. Strain PCC 6803. Appl Environ Microbiol 81, 6857-6863.
 609 Nielsen, J., Fussenegger, M., Keasling, J., Lee, S.Y., Liao, J.C., Prather, K., Palsson, B.,
 610 (2014) Engineering synergy in biotechnology. Nat Chem Biol 10, 319-322.
 611 Nozzi, N.E., Atsumi, S., (2015) Genome engineering of the 2,3-butanediol
 612 biosynthetic pathway for tight regulation in cyanobacteria. ACS Synth Biol 4, 1197-
 613 1204.
 614 Nozzi, N.E., Oliver, J.W., Atsumi, S., (2013) Cyanobacteria as a platform for biofuel
 615 production. Front Bioeng Biotechnol 1, 7.
 616 Oliver, J.W., Atsumi, S., (2014) Metabolic design for cyanobacterial chemical
 617 synthesis. Photosynth Res 120, 249-261.
 618 Oliver, J.W., Atsumi, S., (2015) A carbon sink pathway increases carbon productivity
 619 in cyanobacteria. Metab Eng 29, 106-112.
 620 Oliver, J.W., Machado, I.M., Yoneda, H., Atsumi, S., (2013) Cyanobacterial conversion
 621 of carbon dioxide to 2,3-butanediol. Proc Natl Acad Sci U S A 110, 1249-1254.
 622 Oliver, J.W., Machado, I.M., Yoneda, H., Atsumi, S., (2014) Combinatorial optimization
 623 of cyanobacterial 2,3-butanediol production. Metab Eng 22, 76-82.
 624 Pateraki, I., Andersen-Ranberg, J., Hamberger, B., Heskes, A.M., Martens, H.J., Zerbe,
 625 P., Bach, S.S., Moller, B.L., Bohlmann, J., Hamberger, B., (2014) Manoyl oxide (13R),
 626 the biosynthetic precursor of forskolin, is synthesized in specialized root cork cells
 627 in *Coleus forskohlii*. Plant Physiol 164, 1222-1236.

628 Rabinovitch-Deere, C.A., Oliver, J.W., Rodriguez, G.M., Atsumi, S., (2013) Synthetic
629 biology and metabolic engineering approaches to produce biofuels. Chem Rev 113,
630 4611-4632.

631 Ruffing, A.M., (2013) Borrowing genes from *Chlamydomonas reinhardtii* for free
632 fatty acid production in engineered cyanobacteria. J Appl Phycol 25, 1495-1507.

633 Ruffing, A.M., (2014) Improved Free Fatty Acid Production in Cyanobacteria with
634 *Synechococcus* sp. PCC 7002 as Host. Front Bioeng Biotechnol 2, 17.

635 Salis, H.M., Mirsky, E.A., Voigt, C.A., (2009) Automated design of synthetic ribosome
636 binding sites to control protein expression. Nat Biotechnol 27, 946-950.

637 Sanderson, K., (2011) Lignocellulose: A chewy problem. Nature 474, S12-14.

638 Savakis, P.E., Angermayr, S.A., Hellingwerf, K.J., (2013) Synthesis of 2,3-butanediol
639 by *Synechocystis* sp. PCC6803 via heterologous expression of a catabolic pathway
640 from lactic acid- and enterobacteria. Metab Eng 20, 121-130.

641 Sawangwan, T., Goedl, C., Nidetzky, B., (2010) Glucosylglycerol and
642 glucosylglycerate as enzyme stabilizers. Biotechnol J 5, 187-191.

643 Shen, C.R., Liao, J.C., (2012) Photosynthetic production of 2-methyl-1-butanol from
644 CO₂ in cyanobacterium *Synechococcus elongatus* PCC7942 and characterization of
645 the native acetohydroxyacid synthase. Energy Environ Sci 5, 9574-9583.

646 Tan, X., Du, W., Lu, X., (2015) Photosynthetic and extracellular production of
647 glucosylglycerol by genetically engineered and gel-encapsulated cyanobacteria. Appl
648 Microbiol Biotechnol 99, 2147-2154.

649 Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J.,
 650 Searchinger, T., Somerville, C., Williams, R., (2009) Energy. Beneficial biofuels--the
 651 food, energy, and environment trilemma. *Science* 325, 270-271.
 652 Tirado, M.C., Cohen, M.J., Aberman, N., Meerman, J., Thompson, B., (2010) Addressing
 653 the challenges of climate change and biofuel production for food and nutrition
 654 security. *Food Research International* 43, 1729-1744.
 655 Topp, S., Reynoso, C.M., Seeliger, J.C., Goldlust, I.S., Desai, S.K., Murat, D., Shen, A.,
 656 Puri, A.W., Komeili, A., Bertozzi, C.R., Scott, J.R., Gallivan, J.P., (2010) Synthetic
 657 riboswitches that induce gene expression in diverse bacterial species. *Appl Environ*
 658 *Microbiol* 76, 7881-7884.
 659 Ungerer, J., Tao, L., Davis, M., Ghirardi, M., Maness, P.C., Yu, J.P., (2012) Sustained
 660 photosynthetic conversion of CO₂ to ethylene in recombinant cyanobacterium
 661 *Synechocystis* 6803. *Energy Environ Sci* 5, 8998-9006.
 662 van der Woude, A.D., Angermayr, S.A., Puthan Veetil, V., Osnato, A., Hellingwerf, K.J.,
 663 (2014) Carbon sink removal: Increased photosynthetic production of lactic acid by
 664 *Synechocystis* sp. PCC6803 in a glycogen storage mutant. *J Biotechnol* 184, 100-102.
 665 Varman, A.M., Xiao, Y., Pakrasi, H.B., Tang, Y.J., (2013a) Metabolic engineering of
 666 *Synechocystis* sp. strain PCC 6803 for isobutanol production. *Appl Environ Microbiol*
 667 79, 908-914.
 668 Varman, A.M., Yu, Y., You, L., Tang, Y.J., (2013b) Photoautotrophic production of D-
 669 lactic acid in an engineered cyanobacterium. *Microb Cell Fact* 12.
 670 Varman, A.M., Yu, Y., You, L., Tang, Y.J., (2013c) Photoautotrophic production of D-
 671 lactic acid in an engineered cyanobacterium. *Microb Cell Fact* 12, 117-124.

672 Wang, B., Pugh, S., Nielsen, D.R., Zhang, W., Meldrum, D.R., (2013a) Engineering
673 cyanobacteria for photosynthetic production of 3-hydroxybutyrate directly from
674 CO₂. *Metab Eng* 16, 68-77.

675 Wang, W., Liu, X., Lu, X., (2013b) Engineering cyanobacteria to improve
676 photosynthetic production of alka(e)nes. *Biotechnol Biofuels* 6.

677 Xu, Y., Alvey, R.M., Byrne, P.O., Graham, J.E., Shen, G., Bryant, D.A., (2011) Expression
678 of genes in cyanobacteria: adaptation of endogenous plasmids as platforms for high-
679 level gene expression in *Synechococcus* sp. PCC 7002. *Methods Mol Biol* 684, 273-
680 293.

681 Yao, L., Qi, F., Tan, X., Lu, X., (2014) Improved production of fatty alcohols in
682 cyanobacteria by metabolic engineering. *Biotechnol Biofuels* 7, 94.

683 Yoshino, T., Liang, Y., Arai, D., Maeda, Y., Honda, T., Muto, M., Kakunaka, N., Tanaka,
684 T., (2015) Alkane production by the marine cyanobacterium *Synechococcus* sp.
685 NKBG15041c possessing the alpha-olefin biosynthesis pathway. *Appl Microbiol*
686 *Biotechnol* 99, 1521-1529.

687 Zess, E.K., Begemann, M.B., Pflieger, B.F., (2016) Construction of new synthetic
688 biology tools for the control of gene expression in the cyanobacterium
689 *Synechococcus* sp. strain PCC 7002. *Biotechnol Bioeng* 113, 424-432.

690 Zhang, S., Bryant, D.A., (2015) Biochemical validation of the glyoxylate cycle in the
691 cyanobacterium *Chlorogloeopsis fritschii* Strain PCC 9212. *J Biol Chem* 290, 14019-
692 14030.

693 Zhang, S., Liu, Y., Bryant, D.A., (2015) Metabolic engineering of *Synechococcus* sp.
694 PCC 7002 to produce poly-3-hydroxybutyrate and poly-3-hydroxybutyrate-co-4-
695 hydroxybutyrate. Metab Eng 32, 174-183.
696
697

698 Table 1 Chemical production in cyanobacteria

Product	Titer/ Productivity	Host	Additional carbon source	Reference
2-methyl-1-butanol	0.2 g/L	7942		(Shen and Liao, 2012)
Isopropanol	0.1 g/L	7942		(Hirokawa et al., 2015)
23BDO	2.4 g/L	7942		(Oliver et al., 2013)
23BDO	3 g/L	7942	glucose	(McEwen et al., 2016)
Fatty alcohols	3 mg/gDCW	6803		(Yao et al., 2014)
Ethanol	2.3 g/L	6803		(Luan et al., 2015)
3HB	0.5 g/L	6803		(Wang et al., 2013a)
3HP	0.7 g/L	7942		(Lan et al., 2015)
P3HB	5 % DCW	7002		(Zhang et al., 2015)
P3H4HB	4.5% DCW	7002		(Zhang et al., 2015)
Ethylene	5.7 mL/L/h	6803		(Ungerer et al., 2012)
Ethylene	0.9 mL/L/h	6803	xylose	(Lee et al., 2015)
Heptadecane	4 µg/gDCW	15041c		(Yoshino et al., 2015)
Free fatty acids	0.2 g/L	6803		(Liu et al., 2011)
Sucrose	0.1 g/L	7942		(Ducat et al., 2012)
Sucrose	0.1 g/L	6803		(Du et al., 2013)
GG	0.3 g/L	6803		(Tan et al., 2015)
Squalene	0.7 mg/OD/L	6803		(Englund et al., 2014)
13R-manoyl oxide	0.5 mg/gDCW	6803		(Englund et al., 2015)
Farnesene	70 µg/L/OD/day	7120		(Halfmann et al., 2014)
Isoprene	0.3 mg/gDCW	6803		(Bentley et al., 2014)
				(Angermayr et al.,
Lactic acid	0.8 g/L	6803		2014)
Lactic acid	2.2 g/L	6803	acetate	(Varman et al., 2013b)
Isobutanol	0.3 g/L	6803	glucose	(Varman et al., 2013a)

699

700

Fig. 1 Pyruvate based chemical production

Production of pyruvate derived alcohols and diols from atmospheric CO₂ in cyanobacteria. End products are highlighted in red and are shown with the chemical structure. CB- Calvin-Benson cycle.

Fig. 2 Production of hydroxy acids and alkanes in cyanobacteria
 Production of hydroxy acids and alkanes from acetyl-CoA and the tricarboxylic acid cycle (TCA) cycle in cyanobacteria. End products are highlighted in red and are represented with the chemical structure. CB- Calvin-Benson cycle.

Fig. 3 Chemical production from glycogen in cyanobacteria

Production of sucrose and glucosylglycerol in cyanobacteria from atmospheric CO₂.

End products are highlighted in red and are shown with the chemical structure.

Fig. 4 Cyanobacterial production of isoprenoids

Production of isoprenoids from atmospheric CO₂ in cyanobacteria. End products are highlighted in red and shown with chemical structure.